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Effect of nickel and iron co-exposure on human lung cells

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Abstract

Exposure to ambient air particulate matter (PM) is associated with increased mortality and morbidity in susceptible populations. The epidemiological data also suggest a relationship between PM air pollution and impairment of cardiopulmonary function. The mechanisms that may be responsible for these effects are not fully understood and are likely related to perturbations of cellular and molecular functions. One type of PM, residual oil fly ash (ROFA), is of particular interest. ROFA does not contain much organic material, but does contain relatively high quantities of transition metals, predominantly nickel, vanadium, and iron, as well as black carbon and sulfates. In this study, we investigated the effect of two metals (iron and nickel) on the induction of "hypoxia-like" stress and the production of interleukins (ILs) in minimally transformed human airway epithelial cells (1HAEo$^-$). We found that exposure to soluble nickel sulfate results in the induction of hypoxia-inducible genes and IL-8 production by the 1HAEo$^-$ cells. The simultaneous addition of iron in either ferric or ferrous form and nickel completely inhibited IL-8 production and had no effect on "hypoxia-like" stress caused by nickel, suggesting the existence of two different pathways for the induction "hypoxia-like" stress and IL-8 production. The effect of nickel was not related to the blocking of iron entry into cells since the level of intracellular iron was not affected by co-exposure with nickel. The obtained data indicate that nickel can induce different signaling pathways with or without interference with iron metabolism. Our observations suggest that in some cases the excess of iron in PM can cancel the effects of nickel.

Keywords: ROFA; Nickel; Iron; Ferritin; Hypoxia-inducible genes; IL-8

Introduction

Ambient air pollution particulate matter (PM) is considered to present a serious risk to human health on the basis that elevated levels of PM are linked epidemiologically to increased rates of respiratory and cardiovascular diseases (Pope et al., 2002). PM originates from many different natural and anthropogenic sources. The burning of petroleum-based fossil fuel in power plants results in the emission of residual oil fly ash (ROFA). The exposure to ROFA produces injury of airways and lungs, but the mechanism of ROFA toxicity is not completely understood (Dye et al., 1997). ROFA does not contain significant amounts of organic components, but does contain relatively high quantities of transition metals, Fe, Ni, V, Ca, Mg, mostly in the form of soluble sulfates (Dreher et al., 1997). Three metals, namely, Fe, V, and Ni, are present in the largest quantities, and the amount of each metal can vary depending on the source of the particles. In some ROFA, nickel content could comprise up to 35% of the amount of all metals (Gavett et al., 1997; Hamada et al., 2002), and it was recently shown that it is water-soluble nickel that is responsible for the majority of pulmonary injury caused by ROFA (Kodavanti et al., 1997). Moreover, pulmonary inflammation induced by ROFA was reproduced by intratracheal instillation of a mixture of soluble forms of nickel (Dreher et al., 1997). In rats, 12 days of intratracheal instillation of $NiCl_2$, $NiSO_4$, or Ni_3S_2 resulted in inflammatory and fibrotic response in their lungs (Benson et al., 1986). Acute bronchiolitis was induced in Wistar rats by inhalation of nickel chloride aerosols for 5 days (Ishihara et al., 2002). The chronic exposure of rats to these compounds was also associated with chronic active

Abbreviations: DMOG, dimethyloxalylglycine; DFX, deferoxamine mesylate; FIH, factor inhibiting HIF; 1HAEo, human airway epithelial cells; HIF-1, hypoxia-inducible transcription factor 1; IL-8, interleukin 8; PM, particulate matter; PH, prolyl hydroxylase; IRP, iron regulatory protein; ROFA, residual oil fly ash; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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inflammation, fibrosis, and alveolar macrophage hyperplasia (Dunnick et al., 1989). The proinflammatory response is likely to be related to the nickel-induced production of cytokines. Indeed, the secretion of proinflammatory cytokine interleukin (IL)-8 was shown to be up-regulated by nickel exposure (Barchowsky et al., 2002).

Recently, we reported that exposure to both soluble and insoluble nickel compounds strongly activate a hypoxia-inducible pathway, including hypoxia-inducible transcription factor (HIF-1), and a significant number of known hypoxia-inducible genes, among them NDRG-1/Cap43 and VEGF (Salnikow et al., 1999, 2000a, 2002b). It was shown previously that HIF-1 transcription factor plays a role of master regulator of hypoxia-inducible gene expression (Semenza, 2002). Under normoxic conditions, the HIF-1α protein is short lived since von Hippel Lindau tumor suppressor protein (VHL) captures this protein and targets it for degradation (Maxwell et al., 1999). More recently, the hydroxylation of proline 564 in HIF-1α was found to be critical for interaction of this protein with VHL and subsequent proteosomal degradation (Jaakkola et al., 2001; McNeill et al., 2002). This finding provided important link between activity of prolyl hydroxylase (PH) and the induction and activation of HIF-1α protein. Because the activity of PH depends on the level of oxygen and ferrous iron, it was suggested that it may play a role as an oxygen and iron sensor. Nickel being similar to iron is capable of substituting iron in the enzyme. Nickel is, however, not readily oxidized to state III or IV as iron does. It is conceivable therefore that in the presence of nickel, the PHs will be unable to utilize oxygen and this will cause the hypoxic effect similar to that observed in the absence of oxygen.

Iron is another important PM component and some of the pathological effects after PM inhalation may be due to reactive oxygen species produced by iron-catalyzed reactions. For example, it has been suggested that iron could induce IL-8 production in A549 cells due to reactive oxygen species induction (Smith et al., 2000). Thus, it is clear that either iron or nickel alone can produce biological effects. However, there are very few investigations related to the effects of co-exposure of these two metals.

In this study, we tested the hypothesis that "hypoxia-like" and proinflammatory effects of nickel are due to the interference with iron metabolism. To this end, we investigated the effect of nickel and iron, alone and in combination, on induction of hypoxia-inducible protein NDRG-1/Cap43 and IL-8 production by human lung epithelial 1HAEo$^-$ cells. We found that the exposure to nickel sulfate produces "hypoxia-like" stress, as measured by the induction of hypoxia-inducible protein NDRG-1/Cap43, and significantly induces production of IL-8. Similarly, NDRG-1/Cap43 expression and IL-8 production can be induced by the iron chelator deferoxamine, suggesting that exposure to nickel may interfere with iron metabolism. Additionally, nickel-induced IL-8 production, but not "hypoxia-like" stress can be antagonized by co-exposure with iron. Surprisingly, the co-exposure of iron with nickel does not significantly change ferrous or ferric iron uptake. At the same time, we found that co-exposure with nickel leads to the accumulation of high intracellular levels of nickel, which may exceed those of the iron.

Material and methods

Reagents. $NiSO_4 \cdot 6H_2O$ was obtained from Alfa Aesar (Ward Hill, MA). $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, apo-transferrin was obtained from Sigma (St. Louis, MO), and deferoxamine mesylate was obtained from Calbiochem (La Jolla, CA). Protease inhibitor cocktail was purchased from Roche (Indianapolis, IN). Dimethyloxalylglycine was purchased from Frontier Scientific (Logan, UT).

Cell culture. The 1HAEo$^-$ cell line was obtained from Dr. Gruenert (Gruenert et al., 1995). These cells are simian virus 40 transformed, and have cell-surface markers similar to primary airway basal epithelial cells (Dorscheid et al., 1999). Cells were grown on flasks or 100-mm dishes coated with the mixture of BSA (Invitrogen Corporation, Carlsbad, CA) and Vitrogen (Cohesion, Palo Alto, CA) in minimum essential medium with Earle's modified salts (GIBCO-BRL) containing 10% FCS, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. Cells were used at approximately 80% confluency. For exposure to metals, cells were plated in normal growth medium, which was changed to a serum-free/iron-free RPMI 1640 medium to avoid effects of iron from serum or medium. The incubation in serum-free medium with or without iron for 24 h was not toxic for cells. Exposure to 0.5 mM nickel under these conditions resulted in 75% survival. For exposure to ferric iron, 60 μg/ml apotransferrin was added to serum-free RPMI 1640 medium to facilitate iron delivery.

Western blot analysis. Protein extracts from 1HAEo$^-$ cells were obtained after lysing cells in cell lysis buffer (Cell Signaling, Beverly, MA) for 15 min at 4 °C. All samples were centrifuged in an Eppendorf centrifuge at maximum speed for 10 min at 4 °C, and supernatants were stored at −80 °C until analyzed. Equal loading of protein was assured by prior quantitation using the Bradford assay. Protein aliquots were separated on a 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) minigel and transferred onto PVDF membrane (Roche) using Towbin transfer buffer. Western blot analysis for hypoxia-inducible NDRG-1/Cap43 protein levels was performed using rabbit polyclonal antibody, as described previously (Salnikow et al., 2002a). The antibodies against light chain ferritin (D-18) and DMT-1 (N-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against α-tubulin were purchased from Sigma. Immunoreactive bands were detected using horseradish peroxidase-linked secondary antibody and

enhanced chemiluminescence (Perkin Elmer Life Sciences, Boston, MA).

Each membrane was stripped after probing with specific antibodies and reprobed with antibodies against α-tubulin to assure equal loading. Each experiment has been repeated for at least three times and representative blots are shown in the figures.

Determination of interleukin production. The effect of nickel on secreted IL-8 protein levels was determined using the Quantikine IL-8 Immunoassay (R&D Biosystems, Minneapolis, MN). Briefly, cells were plated to collagen-precoated 24-well plates. Cells were washed 24 h later with PBS and media was changed to serum-free RPMI media. Metals were added 1 h later, and incubation continued for an additional 24 h or as shown in the legends. Conditioned media was removed from treated cells and centrifuged for 10 min at 2000 rpm to remove cells or debris. Protease inhibitors were added, and medium was saved at −80 °C or was immediately incubated at 25 °C for 1 h in microtiter wells precoated with antibodies specific to IL-8. After 1 h, incubation plates were processed according to manufacturer's instructions. All samples were run in quadriplicates. Three independent experiments were carried out with essentially same results. The data were reported as the mean value and SD in ng/10^6 cells for one representative experiment.

Iron and nickel determination using atomic absorption. Iron and nickel determinations were made after the exposed cells were counted, washed with PBS, and saved at −80 °C. For the analysis, the cell pellet was thawed and cells were dissolved in 0.2% HNO_3, vortexed, and the debris was removed by centrifugation for 10 min. The supernatant was analyzed for the presence of Fe or Ni against a certified calibration curve for Ni or Fe using an Atomic Absorption Instrument (Model M 6) with a Graphite Furnace attachment (Model GF 95) with an Autosampler Model (FS 95) (Unicam Atomic Absorption, Franklin, MA) using Zeeman background correction. All standards and sample were run in triplicate. The metal measurements have been done twice. The data were reported as the mean value and SD in ng/10^6 cells.

Results

Exposure to nickel leads to a hypoxic stress

We have recently shown that exposure of human lung adenocarcinoma cells A549 to nickel resulted in induction of "hypoxia-like" stress and strong HIF-1-dependent activation of NDRG-1/Cap43 and VEGF genes (Salnikow et al., 2000a, 2002a). Similarly, here we show that exposure of human airway basal epithelial cells 1HAEo$^-$ to 0.25 or 0.5 mM $NiSO_4$ for 20 h resulted in the induction of NDRG-1/Cap43 (Fig. 1). NDRG-1/Cap43 protein was also induced by the iron chelator deferoxamine (DFX), and by the prolyl-hydroxylase inhibitor dimethyloxalylglycine (DMOG), confirming that this is a hypoxia-inducible gene that can be used as a marker of hypoxic stress induced by nickel.

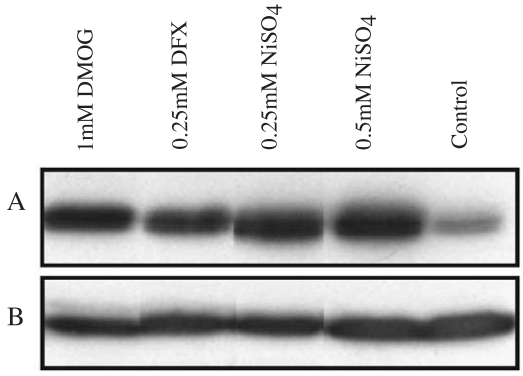

Fig. 1. Nickel exposure causes hypoxic stress in human epithelial airway cells 1HAEo$^-$. To stimulate hypoxic stress, cells were exposed to two different concentrations, $NiSO_4$, or iron chelator DFX, or prolyl-hydroxylase inhibitor DMOG for 20 h. Forty micrograms of total protein was resolved over 10% SDS-PAGE. Western blot analysis was performed using antibodies against: (A) hypoxia-inducible protein NDRG-1/Cap43; (B) α-tubulin.

Exposure to nickel induces secretion of IL-8

Besides the induction of hypoxic stress, exposure to nickel strongly induces secretion of proinflammatory cytokine IL-8. Fig. 2 shows the time course of IL-8 secretion. The amount of IL-8 in the media was tripled after 16 h of exposure and was increased over six times by 48 h. The exposure to DFX also stimulated IL-8 secretion, suggesting that nickel may induce IL-8 secretion through modulation of iron metabolism.

Co-exposure of iron and nickel did not affect iron uptake

Nickel and iron co-exist in ROFA, therefore, lung cells could be exposed to nickel and iron simultaneously. Additionally, because the induction of hypoxic stress by DFX, a strong iron chelator, possibly resulted from the depletion of intracellular iron, it was conceivable that nickel can also interfere with iron metabolism. Ferrous and ferric iron is delivered to cells by two different mechanisms. The delivery of ferrous iron is mediated by the DMT-1 transporter. Nickel can be brought by the same transporter and compete with iron for DMT. Ferric iron is delivered through transferrin/transferrin receptor system. To investigate whether nickel can block entry of ferrous or ferric iron into cells, we co-exposed ferric or ferrous iron in equimolar concentration with nickel in serum-free, iron-free RPMI media and monitored the levels of intracellular iron and nickel. The measurement of intracellular iron by atomic absorption

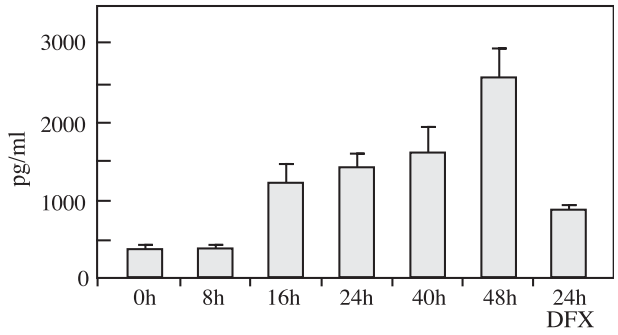

Fig. 2. Time course of Il-8 secretion into the media by 1HAEo$^-$ cells exposed to nickel or DFX for 24 h. 1HAEo$^-$ cells were plated in 24-well plates. After 24 h, media was changed to serum-free RPMI media and cells were exposed to 0.25 mM $NiSO_4$ or 0.25 mM DFX. The amount of secreted IL-8 was detected as described in Material and methods. Data represent mean ± SD.

revealed that the delivery of ferric or ferrous iron was not affected by nickel (Fig. 3A). Surprisingly, a significant amount of nickel was delivered to cells during 24 h co-exposure with iron. When the ratio of nickel to iron was taken, the level of nickel inside the cells was higher than the level of iron (Fig. 3B).

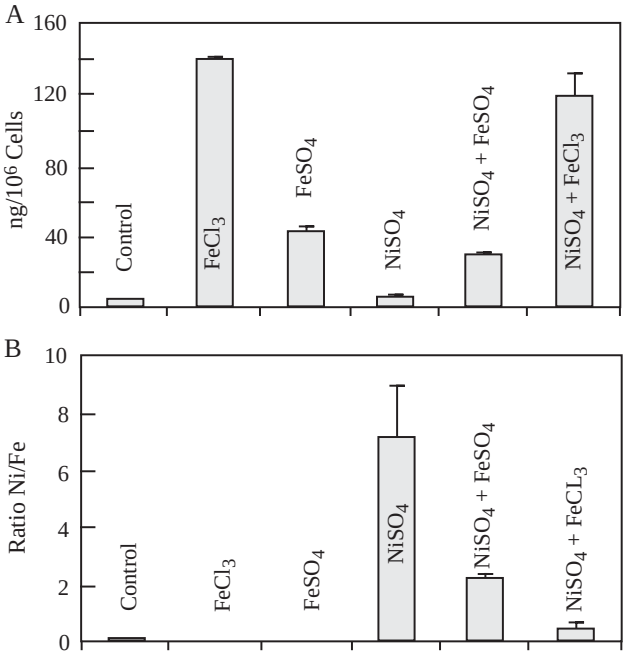

Fig. 3. Intracellular concentration of nickel and iron in 1HAEo$^-$ cells exposed to nickel alone or co-exposed to nickel and ferric or ferrous iron for 24 h. 1HAEo$^-$ cells were plated in 100-mm plates. After 24 h, media was changed to serum-free RPMI media and cells were exposed to 0.25 mM $NiSO_4$ alone or with 0.25 mM $FeSO_4$ or 0.25 mM $FeCl_3$ for another 24 h. The amount of intracellular iron was measured by atomic absorption as described in Material and methods. Data represent mean ± SD. (A) The effect of nickel and iron co-exposure on the intracellular iron level. (B) The ratio of nickel to iron following co-exposure of 1HAEo$^-$ cells to nickel and iron.

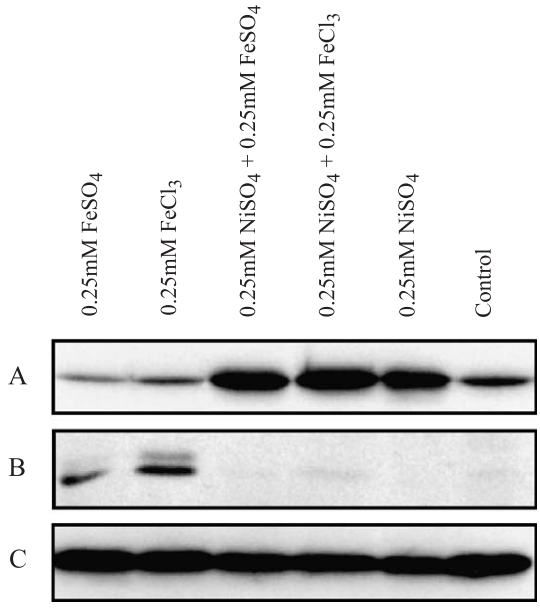

Fig. 4. Co-exposure of iron with nickel does not abrogate hypoxic stress in 1HAEo$^-$ cells. 1HAEo$^-$ cells were co-exposed to 0.25 mM $FeSO_4$ or $FeCl_3$ and 0.25 mM $NiSO_4$ for 20 h. Forty micrograms of total protein was resolved over 12% SDS-PAGE. Western blot analysis was performed on the same membrane using antibodies against: (A) hypoxia-inducible protein NDRG-1/Cap43; (B) ferritin light chain; (C) α-tubulin.

Co-exposure of iron and nickel did not reduce NDRG-1/Cap43 expression, but suppressed IL-8 production

We next investigated whether co-exposure of ferric or ferrous iron with nickel prevented the induction of hypoxia-inducible NDRG-1/Cap43 protein or ferritin production. Fig. 4 shows that the exposure of 1HAEo$^-$ cells to 0.25 mM $FeCl_3$ (ferric) or 0.25 mM $FeSO_4$ (ferrous) alone did not have any effect on NDRG-1/Cap43 protein expression, but caused accumulation of ferritin. However, when iron and nickel were added simultaneously, NDRG-1/Cap43

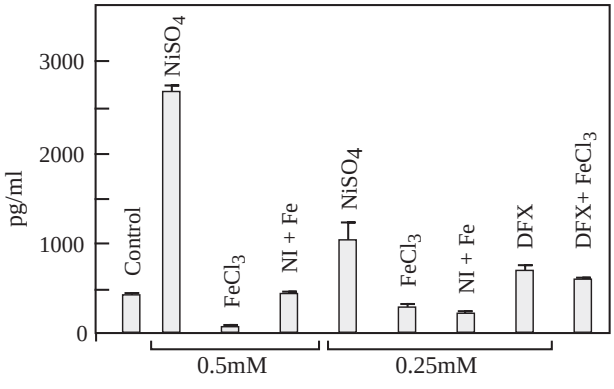

Fig. 5. Effect of iron and nickel co-exposure on IL-8 production. 1HAEo$^-$ cells were plated in 24-well plates. After 24 h, media was changed to serum-free RPMI media and cells were exposed for 24 h to iron or nickel in concentrations indicated on the figure. The amount of secreted IL-8 was detected as described in Material and methods.

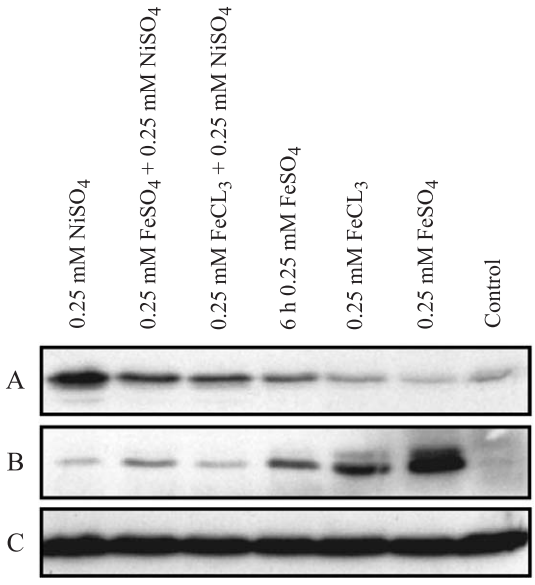

Fig. 6. Pretreatment with iron human 1HAEo$^-$ cells decrease hypoxic stress caused by nickel. Lanes 0.25 mM $FeSO_4$, 0.25 mM $FeCl_3$, or 0.25 mM $NiSO_4$ indicate extracts from cells exposed to metals for 20 h; 6 h 0.25 mM $FeSO_4$ indicate extracts from cells exposed to iron for 6 h. Lanes 0.25 mM $FeCl_3$ + 0.25 mM $NiSO_4$ and 0.25 mM $FeSO_4$ + 0.25 mM $NiSO_4$ indicate extracts from cells pretreated with iron for 6 h following by the addition of nickel for 16 h. Forty micrograms of total protein was resolved over 12% SDS-PAGE. Western blot analysis was done using antibodies against: (A) hypoxia-inducible protein NDRG-1/Cap43; (B) ferritin light chain; (C) α-tubulin.

protein level was induced to the same level as if no iron were added, suggesting that nickel was still inducing the HIF-1-dependent pathway even in the presence of iron. Surprisingly, ferritin protein level dropped significantly (Fig. 4) even when a significant amount of iron was detected by atomic absorption, suggesting that ferritin production was suppressed or iron may be chelated by another ligand.

In contrast, the co-exposure of ferric iron with nickel prevented secretion of IL-8 into media (Fig. 5). Similarly, DFX-induced IL-8 production was suppressed by the addition of iron.

Pretreatment of cells with iron decreases hypoxic stress caused by nickel

To understand how modification of iron metabolism is involved in hypoxic stress, we pretreated cells with 0.25 mM $FeCl_3$ or 0.25 mM $FeSO_4$ for 6 h and then added nickel. The pretreatment of cells with iron resulted in a significant decrease of NDRG-1/Cap43 expression (Fig. 6).

Discussion

Long-term exposure to combustion-related fine particulate air pollution is considered to be an important environmental risk factor for cardiopulmonary and lung cancer mortality (Pope et al., 2002). Numerous studies have attempted to unravel molecular and cellular mechanisms of PM toxicity related to health effects. Heterogeneous composition of particles, namely, metals, black carbon, and sulfates, indicates that PM may contribute to cellular toxicity through a variety of pathways. Size, surface area, and metals content have been considered as important factors contributing to pulmonary toxicity, mainly due to oxidative stress and changes in calcium levels (Brown et al., 2001; Dreher et al., 1997; Dye et al., 1997; Veronesi et al., 2002). However, it became clear recently that soluble transition metals, such as iron, nickel, and vanadium, are responsible for the majority of ROFA toxicity (Dreher et al., 1997; Dye et al., 1997; Gavett et al., 1997; Kodavanti et al., 1997). Iron and vanadium are oxidatively active and Kadiiska et al. (1997) demonstrated that they have a high capacity to generate free radicals, as measured by electron spin resonance. That capacity of nickel was not statistically different from that of saline controls. In agreement with these data, we found that nickel produces very low levels of oxidative stress in human lung A549 cells in vitro and its effects are mostly related to the induction of "hypoxia-like" stress in cells (Salnikow et al., 2000a, 2000b).

Exposure of cells to nickel alone results in the induction and activation of HIF-1 transcription factor and a significant number of HIF-dependent genes including glycolytic enzymes, glucose transporters, and different hydroxylases (Salnikow et al., 1999, 2002b). Among the genes induced by hypoxia, tyrosine hydroxylase is noteworthy (Millhorn et al., 1997) because it is involved in synthesis of catecholamines, which are responsible for the stimulation of heart rate, vasoconstriction, and bronchodilatation. Indeed, it has been shown recently that rats exposed to aerosolized nickel salts demonstrate delayed hypothermia, bradycardia, and arrhythmogenesis (Campen et al., 2001). All these effects are likely to be related to catecholamines.

Here, we investigated the effects of two important PM components, iron and nickel, on the induction of "hypoxia-like" stress and IL-8 production. Exposure of human airway epithelial cells 1HAEo$^-$ to nickel alone for 24 h resulted in strong induction of "hypoxia-like" stress measured by the expression hypoxia-inducible protein NDRG-1/Cap43. The HIF-1-dependent induction of the gene coding for this protein was shown previously using mouse HIF-1α proficient and HIF-1α knockout mouse cells (Salnikow et al., 2000a). We also used two known inducers of hypoxic stress in cells, the iron chelator DFX and the inhibitor of PH DMOG, to show that the level of NDRG-1/Cap43 induction in 1HAEo$^-$ cells by nickel is comparable to that by DFX or DMOG. The levels of nickel and iron used in this in vitro study were in the range of 250–500 μM. In our experiments, we found that the exposure to lower doses of nickel such as 100 μM produces noticeable effect on the induction of hypoxia-inducible genes (not shown). Because all the calculations for nickel exposure were done for $NiSO_4 \cdot 6H_2O$ where nickel itself comprises only 22%, real concentration

of nickel is only one-fifth of the doses used in this study. The amount of nickel found in the lungs of urban dwellers was on average 0.22–1.93 μg/g lung tissue which is roughly 4–30 μM of nickel (Tsuchiyama et al., 1997) and in the lungs of workers in nickel-related industries nickel concentration could be 100 or more times higher (Svenes and Andersen, 1998). These calculations, as well as the fact that the exposure in our study lasted only 20–24 h, as opposed to life-long exposure of general human populations, suggest that doses of metals used in this study were within the range observed for human exposure.

The mechanism of HIF-1 induction by nickel is not known. It is conceivable that nickel is poisoning a recently discovered oxygen sensor, which represents a group of enzymes called hydroxylases. In the presence of oxygen, PH hydroxylates HIF-1α at proline 564 (Ivan et al., 2001; Jaakkola et al., 2001), whereas hydroxylation of asparagine 803 in the C-terminal transactivation domain of HIF-1α by factor inhibiting HIF (FIH) prevents its interaction with acethyltransferase p300 (Lando et al., 2002). PHs and FIH are iron-containing, 2-oxoglutarate-dependent enzymes, and the reaction of hydroxylation requires oxygen, iron, and ascorbate to maintain iron in its ferrous state.

We hypothesized that nickel can either deplete intracellular iron by preventing iron delivery into cells or substitute iron in the hydroxylases. The interaction of iron and nickel uptake has been previously demonstrated. For example, increased nickel absorption in iron-deficient animals has been described (Tallkvist and Tjälve, 1997, 1998; Tallkvist et al., 1994); moreover, the simultaneous addition of nickel impairs ^{59}Fe absorption in a concentration-dependent manner (Schäfer and Forth, 1983). These observations suggest that nickel and iron share the same absorptive pathway. The data have been confirmed by the recent discovery of a divalent metal transporter [DMT1, also called natural resistance-associated macrophage protein 2 (Nramp 2)], which can transport divalent iron and other divalent metals including nickel (Gunshin et al., 1997). It is interesting to note that the DMT1 level was higher in cells without iron, but was not affected by nickel exposure (not shown).

To find the effect of nickel on intracellular iron level, we monitored the level of the major iron-binding protein (ferritin). Exposure to iron in ferrous or ferric form induced ferritin, reflecting an increase in intracellular iron. Because we hypothesize that nickel uptake is mediated by DMT1, it was conceivable that transport of ferrous iron may be affected by nickel co-exposure, whereas transport of ferric iron, mediated by the transferrin receptor system, will be not affected. Surprisingly, co-exposure of nickel and both ferric and ferrous iron led to the disappearance of ferritin. These data indicated that there may be a decrease of intracellular iron level following nickel exposure. The atomic absorption method was used next to directly assess levels of intracellular iron. It was found, however, that following co-exposure of cells with nickel and iron, intracellular iron was practically unchanged, whereas plenty of nickel was inside the cells. Therefore, currently, there are no indications that uptake of nickel and iron is mediated by the same pathway. It was not clear why the co-exposure of cells with nickel and iron leads to the suppression of ferritin production when the intracellular iron level was still high. One possible explanation is that nickel, similar to cobalt and DFX, might block degradation of iron regulatory proteins (IRP) by poisoning 2-oxoglutarate-dependent dioxygenase as described recently (Hanson et al., 2003). Stabilization of IRP2 will block ferritin production even in the presence of iron. Alternatively, nickel exposure induces glucose transport and activates glycolysis. Because glycolysis is occurring in the presence of oxygen, final product of glycolysis pyruvate is converted not into lactate, but rather into citrate. The accumulation of large amounts of citrate could chelate intracellular iron and lead to the disappearance of ferritin in cells co-exposed to iron and nickel. The persistence of "hypoxia-like" stress during the co-exposure of cells with nickel and iron indicates that, either under these conditions, nickel can have preference over iron in iron-containing enzymes, or nickel induces "hypoxia-like" stress via different mechanisms. In case of pretreatment of cells with iron, followed by nickel addition, the induction of NDRG-1/Cap43 protein was, however, alleviated, suggesting that an increase in intracellular iron protected cells from effects of nickel.

Recent studies have indicated that airway epithelial cells also can act as "effector" cells synthesizing and releasing cytokines such as IL-6 and IL-8 in response to many pathologically relevant stimuli, thereby contributing to inflammation (Adler et al., 1994). Acute hypoxia, for example, rapidly and selectively up-regulates IL-8 in macrophages (Hirani et al., 2001). Chronic exposure to nickel also causes lung inflammation, fibrosis, and alveolar macrophage hyperplasia (Dunnick et al., 1989). Nickel exposure induces production of several cytokines such as IL-1β, IL-6, and IL-8 (Nadadur et al., 2000). Secretion of proinflammatory cytokine IL-8 was shown to be up-regulated by nickel exposure, with the activation of a signaling pathway that possibly does not involve the HIF-1 transcription factor (Barchowsky et al., 2002). Here, we show that the exposure to nickel or DFX induces IL-8 secretion. The co-exposure of nickel and iron completely abolished the induction of IL-8. This effect is different from the effect of metal's co-exposure on hypoxic stress, supporting the idea that a different mechanism is involved in up-regulation of IL-8 by nickel. At this point, it is not clear whether induction of other cytokines by nickel is mediated via a pathway similar to IL-8 and can be abolished by iron co-exposure. It also remains to be determined whether iron co-exposed with nickel decreases the risk of pulmonary inflammation caused by nickel in vivo. Several studies have demonstrated that it may be possible. For example, it has been previously shown that the effect of the mixture of iron, vanadium, and nickel on cytokine production was less severe than that caused by nickel alone (Kodavanti et al., 1997). Dreher et al. (1997) have shown that when nickel and vanadium were mixed

together, the injury caused by the mixture was less than the injury caused by nickel alone.

In conclusion, we investigated the effect of two important metal components of ROFA on the induction of "hypoxia-like" stress and cytokine production. We found that the exposure to nickel alone strongly induces hypoxic stress and IL-8 production in human lung 1HAEo$^-$ cells. Co-exposure with iron counteracted the effect of nickel on IL-8 production, but had no effect on hypoxic stress indicating involvement of two different pathways. However, the pretreatment with iron partially protected cells from the hypoxic effects of nickel.

Acknowledgments

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